LESSON 2
CHILL COMA ASSAY AND EVOLUTION INVESTIGATION

VOCABULARY

CHILL COMA: the loss of mobility in insects and other ectotherms at low temperatures

CHILL COMA RECOVERY: the period of time that it takes for an insect to regain mobility after being in a chill coma

ASSAY: investigative (analytic) procedure

EVOLUTION: descent with modification, this includes small-scale evolution (changes in gene frequency in a population from one generation to the next) and large-scale evolution (the descent of different species from a common ancestor over many generations).

NATURAL SELECTION: one of the basic mechanisms of evolution in which differential survival and reproduction of organisms occurs as a consequence of the characteristics of the environment

MUTATION: change in DNA

GENETIC VARIATION: variation in alleles of genes that occurs both within and among populations. Genetic variation is important because it provides the genetic material for natural selection

LESSON SUMMARY

In this two day lesson, with an optional third day exploring the Hardy-Weinberg Principle to quantify evolutionary change in a population, students will have the opportunity to run the Chill Coma Recovery Assay with live Drosophila melanogaster specimens as an engaging introduction before further exploring the mechanisms of evolutionary change in a population, specifically in response to climate change. In Part I of this lesson students will perform a hands on lab procedure; use statistical analysis both on pen and paper, as well as using computer-based spreadsheets in Microsoft Excel; before exploring the mechanisms of evolution via supported self-investigation in Part II. Part III of the lesson is the optional Hardy-Weinberg activity that will further deepen student understanding of biostatistics, including both instruction and practice using the Hardy-Weinberg equations as well as additional application of the Chi-Squared statistical test.

STUDENT LEARNING OBJECTIVES

- SWBAT identify mechanisms of evolution
- SWBAT relate laboratory data to scientific phenomenon
- SWBAT understand the importance of genetic variation in evolution
- SWBAT create graphs and mathematically analyze data collected from an experiment

KEY QUESTION(S):
Is there potential for natural selection to act upon cold coma recovery in Drosophila melanogaster?

OVERALL TIME ESTIMATE:
THREE 45 minute class periods (with optional 4th day for Hardy-Weinberg application)

LEARNING STYLES:
Visual, Kinesthetic, Auditory, Cooperative
CHILL COMA RECOVERY TIME ASSAY BACKGROUND:

The fly *Drosophila melanogaster* has proven to be a successful model for examining organismal thermotolerance and responses to temperature extremes and fluctuations. *Drosophila melanogaster* originated in Africa and ancestral populations were thus presumably adapted to a tropical climate (David and Capy 1988). Beginning approximately 2-3 million years ago, *D. melanogaster* successfully moved out of Africa into a wide variety of climates and habitats, including spreading throughout the north and south temperate zones in both eastern and western hemispheres of the globe (David and Capy 1988). These globally invasive populations have adapted to local environmental conditions and exhibit specific patterns of thermal tolerance and resistance, with tropical species adapted to a warmer climate and temperate species adapted to a more variable climate, as evident from latitudinal clines in allele frequencies at specific genes as well as thermotolerance traits (David and Capy 1988). Several studies have shown evidence that populations in temperate environments display tolerance to high and low temperatures, resulting from seasonal variation in weather conditions, whereas tropical populations are generally intolerant to extreme temperature fluctuations due to the environmental stability of the tropical environment (David and Capy 1988, Hoffman et al. 2003). Studies have shown that there is a significant relationship between latitude and chill-coma recovery time among *Drosophila* species in which tropical species tend to have longer chill-coma recovery times while temperate species have shorter recovery times (Gibert et al. 2001, Overgaard et al. 2011).

At low temperatures, insects and other ectotherms lose their mobility; this is called a chill coma. The temperature at which chill coma is induced varies by species, but for *D. melanogaster*, all populations lose mobility when exposed to 0°C for more than 5 minutes (Gibert et al. 2001). Once brought back to higher temperatures (> 20°C), the insect will recover and resume their normal behavior and activities. Chill-coma recovery time is the period of time that it takes for an insect to regain mobility. A standard assay for measuring chill-coma recovery time is to put vials of flies in ice (0°C) for a certain period of time (e.g., 3 hours) and then measure the length of time it takes for the insect to resume normal behaviors (standing, walking, flying, etc.) (David et al. 1998). This simple assay is valuable to researchers because it requires little in the way of equipment, it can be conducted on large numbers of individuals, and it is useful in assessing differences in thermotolerance between species or between populations of a single species (Overgaard et al. 2011, Sinclair et al. 2012). In the following experiment, students will use this assay to compare chill-coma recovery times in six different lines (3 high resistance/fast recovery lines and 3 low resistance/slow recovery lines) of *Drosophila melanogaster* produced from isofemale lines, which are lines established from separate wild-caught gravid females. Isofemale lines are useful for studying genetic variation because genetic variation becomes fixed within each of these lines but varies clearly between lines, allowing the heritability of particular traits to be measured, analyzed, and compared among isofemale lines with different genetic backgrounds (David et al. 2005). These isofemale lines are derived from the *Drosophila melanogaster* Genetic Reference Panel (Mackay et al. 2012), a set of 192 fully genome-sequenced inbred and genetically homogeneous lines that capture natural genetic variation in cold tolerance and other traits.

Adapted from Classroom Activity: *Illustrating the Concept of Natural Genetic Variation in Traits as a Key Component of Evolution by Natural Selection: Thermotolerance in Drosophila melanogaster flies and Natural Selection in the Face of Contemporary Climate Change*

**MATERIALS**

- Student Page: Chill Coma Assay Procedure and Data Analysis
- Lab Introduction/Background PowerPoint with Teacher Script
- Data Analysis Excel Template
- Chill Coma Lab Materials:
  - 6 Fly Lines — (See details in Hahn Manuscript, referenced below)
  - Petri Dish Halves (tops and bottoms can be used) — 1 dish per pair of students
  - Forceps — 1 per student pair
  - Eppendorf tube (or other small container) of either ethanol or isopropyl alcohol — 1 per pair of students
  - Ice and ice bucket/small cooler
  - Timer/stopwatch
PART II
- Student Page: What Can Cold Flies Tell Us About Evolution?

PART III (EXTENSION)
- Student Page: Measuring Evolution with Hardy-Weinberg

ADVANCE PREPARATION
- Review Background Materials
- Prep Fly Lines, as described in the Hahn Manuscript, referenced below —
  
  **NOTE:** This may take ~2 weeks advanced fly rearing
  
  a. Day of Lab: also prep the necessary number of petri dishes, forceps, and alcohol vials
- Print Student Page: Chill Coma Assay Procedure and Data Analysis
- Print Student Page: What Can Cold Flies Tell Us About Evolution?
- Print Student Page: Measuring Evolution-Hardy-Weinberg Practice Problems

OBTAINING APPROPRIATE DROSOPHILA FOR THE CHILL COMA ASSAY
- **Option One:** Directly order any of the recommended lines (~$15 per stock) from the Bloomington Drosophila Stock Center at Indiana University (http://flystocks.bio.indiana.edu).
- **Option Two:** The laboratory of Dr. Dan Hahn can send you starters of each of the fast and slow recovering stocks if available. Please allow at least two weeks for the starters to be grown and shipped. You can then grow up as many Drosophila as needed for your classes.

The most sustainable option is number one which allows teachers (and students) to work with the handling and rearing of Drosophila. Drosophila are an excellent model organism for many biological and environmental learning activities.

THE CARE AND KEEPING OF DROSOPHILA

Great electronic guide on Drosophila:

The main Drosophila page from Carolina Biological:
http://www.carolina.com/life-science/genetics/drosophila-fruit-fly-genetics/10419.ct

Teachers can easily order instant food and supplies for rearing including bottles, vials, etc. from them. Alternatively, supplies can be purchased from Genesee Scientific (https://genesessci.com).

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES

DAY ONE
1. **(8-10 MINUTES)** Show the provided PPT lesson (using the provided teacher script) to set the stage for the chill coma assay.

2. **(3-5 MINUTES)** Distribute the Student Page: Chill Coma Assay Procedure and Data Analysis
   
   a. Read through the procedure with the students, ensuring they understand the process. A few tips to emphasize when working with the flies:

   i. The students should NOT observe the flies so closely as to be breathing on the dishes (the warmth of their breath will cause the flies to exit the chill coma too quickly)

   ii. A fly is considered “recovered” when it can right itself from its back or side unto all six legs steadily. The fly may take a few steps, but do not let it bump into a neighboring fly, because that might rouse the neighbor too soon.
3. (-20 MINUTES) Pass out fly lines and any other lab materials that students have not collected yet (petri dish, alcohol tube, forceps, timer). Circulate as students perform the assay. NOTE: Some lines may take as long as 20 minutes for all of the flies to recover from the chill coma.

4. (10-15 MINUTES) As students finish collecting the chill coma recovery data instruct them to clean up and begin the data analysis section of the lab packet.

**DAY TWO:**
1. (20-25 MINUTES) Debrief/correct any errors from the individual student data analysis from the previous day and instruct students to enter their fly line data in the provided Data Analysis Excel Template.
   - a. Use the Mean Recovery Time Graph to lead a discussion on variance emphasizing the error bar overlap.

2. (20 MINUTES) Provide students time to answer the related analysis questions in the Student Page: Chill Coma Assay Procedure and Data Analysis.
   - a. (Optional) Debrief the questions as a group and/or collect as a formative assessment.

**DAY THREE**
1. (20-30 MINUTES) Distribute copies of the Student Page: What Can Cold Flies Tell Us About Evolution? to each student and circulate, assisting as necessary, as they read and analyze the information provided about the mechanisms of evolution.

2. (10-15 MINUTES) Debrief and emphasize important points about the mechanisms of evolution. (Use the provided teacher key to assist)

**(OPTIONAL) DAY FOUR**
1. (10-15 MINUTES) Distribute copies of the Student Page: Measuring Evolution with Hardy-Weinberg. Review the background material as a class, providing as many sample practice problems as necessary (see references for further materials on instruction Hardy-Weinberg)

2. (~30 MINUTES) Instruct the students to begin work on the practice problems, circulating and assisting as necessary.

**ASSESSMENT SUGGESTIONS**
- Collect either/all Student Page(s)

**ADDITIONAL TEACHER RESOURCES**
- Chi Squared Instructional Video: “Bozeman Science: Chi-squared Test” https://youtu.be/WXPBoFDqNVk

**REFERENCES**
Hahn, Daniel A. “Classroom Activity: Illustrating the Concept of Natural Genetic Variation in Traits as a Key Component of Evolution by Natural Selection: Thermotolerance in Drosophila melanogaster flies and Natural Selection in the Face of Contemporary Climate Change.” (under review). Available upon request from dahahn@ufl.edu.
**Student Protocol: Chill Coma Assay**

**YOUR MISSION:** Determine whether there is sufficient genetic variation in a population of *Drosophila melanogaster* fruit flies for directional selection of cold tolerance to occur.

**PROCEDURE:**

1. Collect your assigned vials from the ice bucket and/or instructor.

2. Tap your flies into a petri dish (one per line if you were assigned multiple lines) being careful that the flies are not touching one another.
   a. If flies are touching, very gently push them away from one another by using the forceps.

3. Once the flies have been placed in the petri dishes, immediately start your stopwatch.

4. Observe your flies and record the time that each fly recovers on the data sheet. **Recovery is defined as when a fly can stand up on all of its legs.**

5. Once a fly is recovered, immediately pick the fly up with forceps and place it into the provided waste vial (containing ethanol or isopropyl alcohol) so the recovered fly is not accidentally recorded more than once. **Be careful not to touch any of the non-recovered flies**

6. Continue until each fly has been recorded as recovered or is verified as dead by the instructor.
### DATA COLLECTION WORKSHEET

**Vial ID**: 

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<th>Time</th>
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### DATA ANALYSIS

- **Mean**: 
- **Std Dev**: 
- **SEM (95% confidence)**: 

**Upper Error Limit**: 
**Lower Error Limit**: 

*If another group has the same vial ID (and thus fly line) complete your data analysis using the data points from both groups.*
Create an appropriate graph, including 95% confidence error bars, on the axis provided below.

**ANALYSIS QUESTIONS**

1. What type of graph did you construct? Why did you choose this type?

2. Which fly line demonstrated the most rapid recovery time? The slowest recovery time?

3. Which fly lines demonstrated the most significant difference in recovery time? Justify your answer using the lab data.

4. State at least 3 possible sources of error related to data collection in this assay. Suggest a way to correct for each error.
What Can Cold Flies Tell Us About Evolution?

**INTRODUCTION**: From the data you collected in the Chill Coma Assay it is clear that some strains of *Drosophila melanogaster* recover more rapidly from exposure to cold events than others. Considering what you learned about climate change and “whiplash weather” events it appears that it would be advantageous to be a fly from one of the strains that recovers most rapidly. Let’s explore how populations of organisms adapt over time in response to changes in their environment.

By examining the history of life on earth a frequently observed pattern is that organisms with traits that confer an advantage in response to environmental pressures survive and reproduce more frequently than those with less favorable traits. This is known as natural selection and is one of the 5 mechanisms of evolutionary change. The following is a summary of the processes that drive evolution:

First we must consider the question: what is evolution? The most basic definition from a cellular biologist’s perspective is: Evolution is a change in gene (allele) frequencies over time.

What drives these changes?

![5 Agents of Evolutionary Change](image)

While all of these agents of change drive evolution, we will focus on natural selection. For natural selection to occur the following conditions must be met:

1. There must be variation within the population.
2. This variation is genetically inherited
3. This variation leads to differential reproduction
LET'S REVIEW WHAT YOU JUST LEARNED:

1. Explain what it means for genetic variation to be present in a population.

2. Which population has more genetic variation? Explain your answer.
   Population 1: A research strain of flies which are inbred specifically for the homozygous recessive white eye color allele or
   Population 2: Wild type fruit flies collected from a North Carolina Farmer’s market.

3. Explain differential reproduction in your own words.

4. Why is it important that variation be heritable?

WHAT CAUSES THIS GENETIC VARIATION IN THE FIRST PLACE?

GOOD QUESTION. That’s where the other 4 agents of change come into play, resulting in various allele frequencies within an interbreeding population (species).

• **MUTATIONS** cause changes to the DNA (the alleles) and possibly to the expressed protein and thus phenotype of the organism.

• **GENE FLOW** increases variation when organisms emigrate into a new area or immigrate out of an existing one, thus changing the allele frequency of that population’s gene pool.

• **NON-RANDOM MATING** (or sexual selection) - Sexual selection acts on an organism’s ability to obtain, or successfully copulate, with a mate. Those individuals who successfully mate more often, due to their desirable sexual traits (a thick, bushy mane on a male lion or long, brilliant tail feathers of a male peacock) will pass on the alleles for these sex related genes in greater frequency than those individuals who have less “attractive” sex features and thus reproduce less.

• **GENETIC DRIFT** – alternations in the allele frequencies of a population due to change. No selective pressure is involved in genetic drift; it is due to random chance and occurs in all populations.
Well, when you consider all the agents of evolutionary change you are analyzing an organism’s fitness. Fitness is a handy concept because it lumps everything that matters to natural selection (survival, mate-finding, reproduction) into one idea. **THE FITTEST INDIVIDUAL IS NOT NECESSARILY THE STRONGEST, FASTEST, OR BIGGEST.** A genotype’s fitness includes its ability to survive, find a mate, produce offspring — and ultimately leave its genes in the next generation.

**YOU TRY IT:**

1. Would it be possible for a population of flies with no mutation, gene flow, non-random mating, or genetic drift to undergo natural selection? Explain why or why not.

2. Do the following examples best describe mutation, gene flow, nonrandom mating or genetic drift?
   a. Female hornbills prefer males with larger beaks as mates. ____________
   b. The DNA of a fingered tree frog changes as a result of exposure to UV radiation from the sun. ____________
   c. A few individuals of common Coquí are isolated from the larger population by a new road (a chance event). As a result the allele frequencies in the small isolated population begin to change significantly from the allele frequencies in the original larger population. ____________
   d. Asian tiger mosquitoes are expanding their ranges. As a result individuals are frequently leaving a population to travel further north. These individuals do not return to the original population. ____________

3. Assuming that all the traits contributing to the results below are genetically controlled, which member of a population of Fantail Warblers is most evolutionarily fit? One that ____________
   a. lives 12 years, produces 36 eggs, 19 hatch and reproduce.
   b. lives 14 years, produces 26 eggs, 14 hatch and reproduce.
   c. lives 7 years, produces, 24 eggs, 21 hatch and reproduce.
   d. lives 5 years, produces 23 eggs, 18, hatch and reproduce.
   e. lives 17 years, produces 20 eggs, 20 hatch and reproduce.

Explain your answer:
**THINKING BACK TO THE LAB DATA:**

1. Given what you now know about evolution, propose why the different strains of flies you observed have different chill coma recovery times.

2. How can you tell if there is genetic variation within this population using the results from your assay?

3. If you were going conduct future research, which of the lines you conducted the assay on would you be most interested in studying and why?

4. Why is it likely that chill coma recovery time would be a trait that natural selection acts upon?
5. You explored 6 different lines of flies, but in reality there are nearly 600 lines. 40 are displayed below.

![Graph showing chill coma recovery time by line and sex](image)

- a. Given the above information, why is collaboration important in science?

- b. Using the complete data set provided above suggest an additional research question you could investigate.

6. While you observed differences in chill coma recovery time, the exact mechanism is currently unknown. Use your knowledge of experimental design to write a procedure investigating one of the following:

- a. The effect of different times that flies are kept on ice to assess how varying the time of exposure to physiological stress influences recovery time.

- b. The role of age on recovery times to evaluate how organisms differ in their ability to handle physiological stress as they age.

- c. The role of developmental temperature on the expression of cold tolerance to determine if rearing temperature affects later resistance to cold stress.
**Measuring Evolution With The Hardy-Weinberg Principle**

**INTRODUCTION:** We have examined genetic variation within one species of Drosophila (fruit fly). Could this genetic drive evolution and ultimately speciation? (the lineage-splitting event that produces two or more separate species). Is there a way to quantify these genetic changes through time? The answer to both of the above questions is “YES!” thanks to the widely used Hardy-Weinberg Principle. Read the following to investigate more about this principle:

**THE HARDY-WEINBERG PRINCIPLE**

Developed in the early 20th century by Godfrey Hardy, a mathematician, and Wilhelm Weinberg, a physician, the Hardy-Weinberg equations serve as a null hypothesis model; meaning that if a population’s allele frequencies equal the variables in the Hardy-Weinberg equations the population is NOT experiencing any changes in allele frequencies and thus is not undergoing evolution. This population would be referred to as being in Hardy-Weinberg Equilibrium.

To be in Hardy-Weinberg Equilibrium, a population must exhibit the following conditions:

1. very large population size (no genetic drift)
2. no migration (no gene flow in or out)
3. no mutation (no genetic change)
4. random mating (no sexual selection)
5. no natural selection (every genotype is equally fit)

The Hardy-Weinberg Principle consists of two equations: one applies directly to alleles, while the other is used to measure genotypes:

\[ p + q = 1 \]
\[ p^2 + 2pq + q^2 = 1 \]

- \( p \) = frequency of the dominant allele
- \( q \) = frequency of the recessive allele
- \( p^2 \) = frequency of the homozygous dominant genotype
- \( 2pq \) = frequency of the heterozygous genotype
- \( q^2 \) = frequency of the homozygous recessive genotype
GREAT! SO, WHAT DOES THAT MEAN FOR AN ACTUAL POPULATION OF ORGANISMS?

Thus, if the allele frequencies of particular gene are measured in a population, using the Hardy-Weinberg equations we can determine if the population is in Hardy Weinberg Equilibrium for that particular gene (aka, not evolving) or if the population is NOT in equilibrium, then it is exhibiting signs of evolution, due to as evidenced by a change in the allele frequency. This can be accomplished by comparing the allele values calculated using the Hardy-Weinberg equations to the allele frequencies measured in the wild population.

For example if the calculated frequency of the p allele (dominant allele) is determined to be 0.7 for a given gene in a particular population, and the calculated frequency of the q allele is 0.3 for the same gene, then $0.7 + 0.3 = 1$ at one generation. If the same allele frequencies are observed in the population in future generations then this population is in Hardy-Weinberg equilibrium; therefore the population does not have significant changes in allele frequency (compared to the calculated values) for that gene and thus is not evolving. However, if the allele frequencies change substantially from one generation to another generation in the future (one or more generations later), then evolution is occurring in that population. Although many people think of evolution as only a slow process, it can occur quickly in some circumstances. For example, evolution can happen in as little as 1-2 generations!

READY TO PUT THE PRINCIPLE TO WORK?

The exact genetic mechanism for recovery from the chill coma phenomenon is not yet known in Drosophila melanogaster, however for the purposes of this exercise, and all the following practice problems, let’s assume it’s controlled by a single, completely dominant gene named Chilly. The dominant allele displays rapid chill coma recovery time and homozygous recessive individuals display a slower chill coma recovery.

1. In a sampled population of Drosophila melanogaster of 250 individuals, 192 recover from chill coma rapidly. Determine the p and q values for this population.

2. In the same population described in question one, what percentage of flies are heterozygous for the Chilly gene?

3. A larger population of Drosophila melanogaster is sampled and out of 2020 individuals, 392 recover slowly from chill coma. Determine the number of individuals that are homozygous dominant, heterozygous and homozygous recessive.

4. In a small population of flies the expected allele frequencies are $p = 0.6$ and $q = 0.4$. When the population is analyzed the actual frequencies observed are $p = 0.8$ and $q = 0.2$. Give at least two possible reasons why the observed and expected frequency did not match.
5. As described in question 4, often the expected and observed allele frequencies DO NOT match, since the Hardy-Weinberg equilibrium equations serve only as a null hypothesis. Further statistical analysis must be done to determine if the differences between observed and expected values are significant (p value of 0.5 or less) enough to warrant further investigation. Run a Chi Squared analysis (a chart has been provided to help you organize the analysis), given the following information to determine if the population of flies should be more closely studied for evidence of evolution.

   a. In a population of 300 flies, 260 display the dominant phenotype. Determine the number of expected individuals for all three genotypes and record them in the table below.

   b. The population of flies is sampled and the p value is determined to be 0.9. Determine the number of observed individuals for all three genotypes and record them in the table below.

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\[ X^2 = \]

   c. How many degrees of freedom do you have? _______________

   d. Use the Chi Squared Table below to determine the P value. _______________

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   e. Interpret the P value as it relates to these data. Explain the significance in terms of evolution.
**INTRODUCTION:** From the data you collected in the Chill Coma Assay it is clear that some strains of *Drosophila melanogaster* recover more rapidly from exposure to cold events than others. Considering what you learned about climate change and “whiplash weather” events it appears that it would be advantageous to be a fly from one of the strains that recovers most rapidly. Let’s explore how populations of organisms adapt over time in response to changes in their environment.

By examining the history of life on earth a frequently observed pattern is that organisms with traits that confer an advantage in response to environmental pressures survive and reproduce more frequently than those with less favorable traits. This is known as natural selection and is one of the 5 mechanisms of evolutionary change. The following is a summary of the processes that drive evolution:

First we must consider the question: what is evolution? The most basic definition from a cellular biologist’s perspective is: Evolution is a change in gene (allele) frequencies over time.

What drives these changes?

While all of these agents of change drive evolution, we will focus on natural selection. For natural selection to occur the following conditions must be met:

1. There must be variation within the population.
2. This variation is genetically inherited
3. This variation leads to differential reproduction
LET’S REVIEW WHAT YOU JUST LEARNED:

1. Explain what it means for genetic variation to be present in a population.

*Individuals within the population will have different allele frequencies, which may or may not display as differences in phenotype as well. AKA the individuals ARE NOT genetically identical.*

2. Which population has more genetic variation? Explain your answer.
   - Population 1: A research strain of flies which are inbred specifically for the homozygous recessive white eye color allele.
   - Population 2: Wild type fruit flies collected from a North Carolina Farmer’s market.

*Population 2, because we are assuming they would not be genetically identical, since they were collected from the wild and are not manipulated in the laboratory.*

3. Explain differential reproduction in your own words.

*Some individuals have greater reproductive success than others; those individuals with greater reproductive success will contribute more of their genes to gene pool of the following generations.*

4. Why is it important that variation be heritable?

*It is important that the genetic variation (especially those traits that are advantageous) be passed on to the next generation (or inherited).*

**WHAT CAUSES THIS GENETIC VARIATION IN THE FIRST PLACE?**

**GOOD QUESTION.** That’s where the other 4 agents of change come into play, resulting in various allele frequencies within an interbreeding population (species).

- **Mutations** cause changes to the DNA (the alleles) and possibly to the expressed protein and thus phenotype of the organism.

- **Gene Flow** increases variation when organisms emigrate into a new area or immigrate out of an existing one, thus changing the allele frequency of that population’s gene pool.

- **Non-random mating** (or sexual selection)- Sexual selection acts on an organism’s ability to obtain, or successfully copulate, with a mate. Those individuals who successfully mate more often, due to their desirable sexual traits (a thick, bushy mane on a male lion or long, brilliant tail feathers of a male peacock) will pass on the alleles for these sex related genes in greater frequency than those individuals who have less “attractive” sex features and thus reproduce less.

- **Genetic Drift** – alternations in the allele frequencies of a population due to change. No selective pressure is involved in genetic drift; it is due to random chance and occurs in all populations.
GREAT! SO....WHAT DOES ALL THIS MEAN?

Well, when you consider all the agents of evolutionary change you are analyzing an organism’s fitness. Fitness is a handy concept because it lumps everything that matters to natural selection (survival, mate-finding, reproduction) into one idea. **THE FITTEST INDIVIDUAL IS NOT NECESSARILY THE STRONGEST, FASTEST, OR BIGGEST.** A genotype’s fitness includes its ability to survive, find a mate, produce offspring — and ultimately leave its genes in the next generation.

YOU TRY IT:

1. Would it be possible for a population of flies with no mutation, gene flow, non-random mating, or genetic drift to undergo natural selection? Explain why or why not.

   *No, because the described population above is exhibiting all the traits required for Hardy-Weinberg equilibrium and would not be experiencing evolutionary events. (OR: There would not be any genetic variation for natural selection to act upon.)*

2. Do the following examples best describe mutation, gene flow, nonrandom mating or genetic drift?

   a. Female hornbills prefer males with larger beaks as mates. **Nonrandom mating**

   b. The DNA of a fingered tree frog changes as a result of exposure to UV radiation from the sun. **Mutation**

   c. A few individuals of common Coquí are isolated from the larger population by a new road (a chance event). As a result the allele frequencies in the small isolated population begin to change significantly from the allele frequencies in the original larger population. **Genetic drift**

   d. Asian tiger mosquitoes are expanding their ranges. As a result individuals are frequently leaving a population to travel further north. These individuals do not return to the original population. **Gene flow**

3. Assuming that all the traits contributing to the results below are genetically controlled, which member of a population of Fantail Warblers is most evolutionarily fit? One that ____________

   a. lives 12 years, produces 36 eggs, 19 hatch and reproduce.

   b. lives 14 years, produces 26 eggs, 14 hatch and reproduce.

   c. **lives 7 years, produces, 24 eggs, 21 hatch and reproduce.**

   d. lives 5 years, produces 23 eggs, 18, hatch and reproduce.

   e. lives 17 years, produces 20 eggs, 20 hatch and reproduce.

   Explain your answer:

   *The bird described in choice c has the most reproductively successful offspring, thus its alleles are more frequently found in the gene pool.*
THINKING BACK TO THE LAB DATA:

1. Given what you now know about evolution, propose why the different strains of flies you observed have different chill coma recovery times.

*If the chill coma response phenomenon is genetic, then the different lines represent genetic variation for that trait.*

2. How can you tell if there is genetic variation within this population using the results from your assay?

*Based on the statistical analysis, those lines with significant genetic variation will NOT show any overlap in their 95% confidence error bars.*

3. If you were going conduct future research, which of the lines you conducted the assay on would you be most interested in studying and why?

*Student answers will vary, but ideally students will chose the line with the fastest response time, since those individuals would likely survive current climate change patterns more successfully than a slow recovery line.*

4. Why is it likely that chill coma recovery time would be a trait that natural selection acts upon?

*Fly populations that have a rapid chill coma recovery time would be more likely to survive rapidly changing climates/whiplash weather events and thus experience differential reproduction, passing on the advantageous alleles to the next generation, compared to a population with slow chill coma recovery time.*
5. You explored 6 different lines of flies, but in reality there are nearly 600 lines. 40 are displayed below.

   ![Graph showing Chill coma recovery time by line and sex]

   a. Given the above information, why is collaboration important in science?

   *Student answers will vary, but ideally they will discuss the large number of lines, the logistics of such a large study, etc.*

   b. Using the complete data set provided above suggest an additional research question you could investigate.

   *Student answers will vary but may include:*

   Why is there a difference between male and females of the same line?

   Why is line 517 so different from the other lines?

6. While you observed differences in chill coma recovery time, the exact mechanism is currently unknown. Use your knowledge of experimental design to write a procedure investigating one of the following:

   a. The effect of different times that flies are kept on ice to assess how varying the time of exposure to physiological stress influences recovery time.

   b. The role of age on recovery times to evaluate how organisms differ in their ability to handle physiological stress as they age.

   c. The role of developmental temperature on the expression of cold tolerance to determine if rearing temperature affects later resistance to cold stress.

   *Student answers will vary, but should include clear IV and DV, controls, constants, replication, duration of experiment, data collection consideration, etc.*
INTRODUCTION: We have examined genetic variation within one species of Drosophila (fruit fly). Could this genetic drive evolution and ultimately speciation? (the lineage-splitting event that produces two or more separate species). Is there a way to quantify these genetic changes through time? The answer to both of the above questions is “YES!” thanks to the widely used Hardy-Weinberg Principle. Read the following to investigate more about this principle:

THE HARDY-WEINBERG PRINCIPLE

Developed in the early 20th century by Godfrey Hardy, a mathematician, and Wilhelm Weinberg, a physician, the Hardy-Weinberg equations serve as a null hypothesis model; meaning that if a population’s allele frequencies equal the variables in the Hardy-Weinberg equations the population is NOT experiencing any changes in allele frequencies and thus is not undergoing evolution. This population would be referred to as being in Hardy-Weinberg Equilibrium.

To be in Hardy-Weinberg Equilibrium, a population must exhibit the following conditions:

1. very large population size (no genetic drift)
2. no migration (no gene flow in or out)
3. no mutation (no genetic change)
4. random mating (no sexual selection)
5. no natural selection (every genotype is equally fit)

The Hardy-Weinberg Principle consists of two equations: one applies directly to alleles, while the other is used to measure genotypes:

\[ p + q = 1 \]
\[ p^2 + 2pq + q^2 = 1 \]

- \( p \) = frequency of the dominant allele
- \( q \) = frequency of the recessive allele
- \( p^2 \) = frequency of the homozygous dominant genotype
- \( 2pq \) = frequency of the heterozygous genotype
- \( q^2 \) = frequency of the homozygous recessive genotype
Thus, if the allele frequencies of particular gene are measured in a population, using the Hardy-Weinberg equations we can determine if the population is in Hardy Weinberg Equilibrium for that particular gene (aka, not evolving) or if the population is NOT in equilibrium, then it is exhibiting signs of evolution, due to as evidenced by a change in the allele frequency. This can be accomplished by comparing the allele values calculated using the Hardy-Weinberg equations to the allele frequencies measured in the wild population.

For example if the calculated frequency of the p allele (dominant allele) is determined to be 0.7 for a given gene in a particular population, and the calculated frequency of the q allele is 0.3 for the same gene, then $0.7 + 0.3 = 1$ at one generation. If the same allele frequencies are observed in the population in future generations then this population is in Hardy-Weinberg equilibrium; therefore the population does not have significant changes in allele frequency (compared to the calculated values) for that gene and thus is not evolving. However, if the allele frequencies change substantially from one generation to another generation in the future (one or more generations later), then evolution is occurring in that population. Although many people think of evolution as only a slow process, it can occur quickly in some circumstances. For example, evolution can happen in as little as 1-2 generations!

The exact genetic mechanism for recovery from the chill coma phenomenon is not yet known in *Drosophila melanogaster*, however for the purposes of this exercise, and all the following practice problems, let’s assume it’s controlled by a single, completely dominant gene named Chilly. The dominant allele displays rapid chill coma recovery time and homozygous recessive individuals display a slower chill coma recovery.

1. In a sampled population of *Drosophila melanogaster* of 250 individuals, 192 recover from chill coma rapidly. Determine the p and q values for this population.

   \[
   q^2 = \frac{58}{250} \quad q = 0.48 \\
   p = 0.52
   \]

2. In the same population described in question one, what percentage of flies are heterozygous for the Chily gene?

   \[
   \text{Heterozygous} = 2pq = 2(0.52)(0.48) = 0.50
   \]

3. A larger population of *Drosophila melanogaster* is sampled and out of 2020 individuals, 392 recover slowly from chill coma. Determine the number of individuals that are homozygous dominant, heterozygous and homozygous recessive.

   \[
   q^2 = \frac{392}{2020} \quad \text{homo dom} = p^2 = (0.56)^2 \times 2020 = 633 \text{ individuals} \\
   \text{hetero dom} = 2pq = 2(0.56)(0.44) \times 2020 = 995 \text{ individuals} \\
   q = 0.44 \quad \text{homo rec} = q^2 = (0.44)^2 \times 2020 = 372 \text{ individuals} \\
   p = 0.56
   \]

4. In a small population of flies the expected allele frequencies are $p = 0.6$ and $q = 0.4$. When the population is analyzed the actual frequencies observed are $p = 0.8$ and $q = 0.2$. Give at least two possible reasons why the observed and expected frequency did not match.

   *Student answers will vary. Any of the above “Hardy-Weinberg Conditions” and/or the population is exhibiting evolution via changes in allele frequency are acceptable answers.*
5. As described in question 4, often the expected and observed allele frequencies DO NOT match, since the Hardy-Weinberg equilibrium equations serve only as a null hypothesis. Further statistical analysis must be done to determine if the differences between observed and expected values are significant (p value of 0.5 or less) enough to warrant further investigation. Run a Chi Squared analysis (a chart has been provided to help you organize the analysis), given the following information to determine if the population of flies should be more closely studied for evidence of evolution.

a. In a population of 300 flies, 260 display the dominant phenotype. Determine the number of expected individuals for all three genotypes and record them in the table below.

\[ q^2 = \frac{40}{300} \quad \text{homo dom} = p^2 = (0.63)2 \times 300 = 119 \text{ individuals} \]
\[ p = 0.63 \quad \text{homo rec} = q^2 = (0.37)2 \times 300 = 41 \text{ individuals} \]
\[ q = 0.37 \quad \text{hetero dom} = 2pq = 2(0.63)(0.37) \times 300 = 140 \text{ individuals} \]

b. The population of flies is sampled and the p value is determined to be 0.9. Determine the number of observed individuals for all three genotypes and record them in the table below.

\[ p = 0.90 \quad \text{homo dom} = p^2 = (0.90)2 \times 300 = 242 \text{ individuals} \]
\[ q = 0.10 \quad \text{hetero dom} = 2pq = 2(0.90)(0.10) \times 300 = 54 \text{ individuals} \]
\[ \text{homo rec} = q^2 = (0.10)2 \times 300 = 3 \text{ individuals} \]

<table>
<thead>
<tr>
<th>Genotype/Phenotype</th>
<th>Observed (o)</th>
<th>Expected (e)</th>
<th>(o-e)</th>
<th>(o-e)^2/e</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC / quick recovery</td>
<td>242</td>
<td>119</td>
<td>124</td>
<td>129.2</td>
</tr>
<tr>
<td>Cc / quick recovery</td>
<td>54</td>
<td>140</td>
<td>-86</td>
<td>52.8</td>
</tr>
<tr>
<td>cc / slow recovery</td>
<td>3</td>
<td>41</td>
<td>-38</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(X^2 = 217.2)</td>
</tr>
</tbody>
</table>

c. How many degrees of freedom do you have? 2

d. Use the Chi Squared Table below to determine the P value. less than 0.01

<table>
<thead>
<tr>
<th>CHI-SQUARE TABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degrees of Freedom</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>0.05</td>
</tr>
<tr>
<td>0.01</td>
</tr>
</tbody>
</table>

e. Interpret the P value as it relates to these data. Explain the significance in terms of evolution.

A p value of less than 0.01 means that there is less than a 1% probability that the difference in observed vs expected is due solely to random chance. Thus we can assume that this population is NOT in Hardy-Weinberg equilibrium and is undergoing changes in allele frequency due to evolution events.
Notes: